The NIH rDNA Guidelines Explained

The next few pages were written to extract the essence of the NIH guideline requirements and put them into readable form. Since many details are omitted, the actual guidelines should be consulted when in doubt (http://www4.od.nih.gov/oba/rac/guidelines/guidelines.html). Plant, whole animals, and human experiments are given special treatment in the guidelines (and in this explanation).

Prerequisites

To make sense of the following, the reader should know the basic ideas behind Biosafety Levels 1 through 4. A summary table giving the characteristics of the four laboratory Biosafety Levels is on page 6. Animal Biosafety levels are summarized on page 7.

What Is Recombinant DNA (rDNA)?

It is either a) DNA constructed *in vitro* from separate DNA segments that can replicate and/or express a biologically active polynucleotide or polypeptide *in vivo*, or b) synthetic DNA that has the potential of generating a hazardous product *in vivo.*

What Are the RAC and the OBA?

The NIH OBA (Office of Biotechnology Activities) is an administrative arm responsible for carrying out the orders of the NIH Director with regard to recombinant DNA, genetic testing, and xenotransplantation. An advisory committee is involved in establishing policies for each of these fields. For recombinant DNA the committee is called the Recombinant DNA Advisory Committee or the "RAC".

Registration

The NIH requires all labs working with recombinant DNA to register with their local Institutional Biosafety Committee (IBC). The guidelines distinguish among the five kinds of registrations. The type depends on the potential hazard of the work. More hazardous means more approvals are needed. The five types are:

- Work that cannot begin until there is NIH and IBC approval (NIH Guidelines Sections III-A and III-B), this is the most dangerous level and it is [TABOO] (page 2)
- Work that cannot begin until there is IBC and RAC review (Sections III-C). [WAIT and WAIT] (page 2) This tends to be sensitive and potentially dangerous. Human Gene Transfer studies are in this category (page5).
- Work that cannot begin until there is IBC approval (III-D), there is usually a short [WAIT] (page 2).
- Work can begin when the IBC is notified (Section III-E) [NO WAITING] (page 4)
- Work that is Exempt form NIH Guidelines (Section III-F). No approval is needed but the work should be registered with the IBC [**NO WAITING, EXEMPT**] (page 4)

"TABOO"

(NIH approval and IBC Approval Required)

(from NIH Guidelines Sections III-A and III-B)

- Making drug-resistant constructs of microorganisms if they compromise the drug's therapeutic potential
- Making constructs that synthesize vertebrate toxins with an LD50 of 100ng/Kg or less.

- Making constructs in *E. coli* that synthesize vertebrate toxins that are "lethal" between 100ng/Kg and 100µg/Kg

"WAIT AND WAIT"

(NIH Review and IBC Approval Required)

(from Section IIIC)

Studies in which genes are transferred into humans must be submitted to the NIH OBA for review. If the OBA finds the study to be "novel," it will place the study on the next RAC meeting agenda. IBC approval must wait for the RAC's review. If, on the other hand, OBA does not deem the study to be "novel," IBC can act immediately.

"WAIT"

(IBC Approval Needed Before Starting)

These studies are examined by the IBC with an eye to recommending safe procedures and containment. To reach a conclusion it is often useful to classify the risk associated with a proposed study according to the risk associated with the organism(s) to be used. A convenient classification tool is the concept of "risk group".

Risk Groups

The NIH classifies biological agents into four Risk Groups according to their human pathogenecity (see NIH Guidelines, Section II-A-1)

- Risk Group 1 – not associated with disease in healthy adults
- Risk Group 2 – associated with disease that is rarely serious and for which therapeutic or preventive options are often available
- Risk Group 3 - associated with serious or lethal disease for which therapeutic or preventive options may be available
- Risk Group 4 – associated with serious or lethal disease for which therapeutic or preventive options *not usually* available

Appendix B in the NIH Guidelines lists a number of biologic agents according to their Risk Group.

In general, the Risk Group determines the Biosafety Level needed: for instance a Risk Group 3 agent is usually studied in a BL3 or BL3-N (animal) or BL3-P (plants) lab.

Those agents not listed in Risk Group (RGs) 2, 3 and 4 are not automatically or implicitly I RG1: a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

The table on the next page summarizes the NIH recommendations for Biosafety Level according to risk group.

"NO WAITING"

Just Notify IBC Before Work Starts

Notification is necessary because all recombinant DNA studies must be registered with Clemson University.

These studies involve "Low Hazard" recombinant DNA (Section III-E)

- Non-pathogenic prokaryotes or non-pathogenic lower eukaryotes (use BL1)
- Recombinant DNA with less than 2/3 of a eukaryotic viral genome (and no helper) used exclusively in tissue culture (BL1 recommended)

- Some host plants carrying rDNA. BL1-P level containment is recommended when the host is:
    - a non-noxious weed,
    - a plant or microorganism thought not to damage the ecosystem,
- Some other host plants carrying recombinant DNA. BL3-P or BL1-P+ level containment is recommended when:
    - the host is a noxious weed,
    - the introduced DNA is the complete genome of an infectious agent known to normally exist in the United States
    - the host is a plant or microorganism that may damage ecosystems,
    - the host is a plant with recombinant DNA from foreign microorganisms that is thought to be safe for the ecosystem.

No Waiting - Exempt

Exempt Recombinant DNA (Section III-F and Appendix C)

"Exempt" form NIH guidelines means that work with these constructs need not be approved by the IBC. However, Clemson University requires that all recombinant work, exempt or non-exempt, be registered. Thus, it is Clemson University policy to insist that investigators using any rDNA register with the IBC. The exempt work can be performed at BL1.

• Gene transfer into Human subjects (RAC review process[1] is required before IBC can approve this kind of study). (Section III-C-1)	
• Recombinant organisms cultured in volumes greater than 10 liters (Section III-D-6).	
• Pathogen Hosts (Section III-D-1): Check Appendix B to determine their Risk Group - then:	
	Risk Group 2 host. BL2, BL2-N
	Risk Group 3 host. BL3, BL3-N
	Risk Group 4 host. BL4, BL4-N
• Pathogen DNA Source into Non-Pathogen Host (Section III-D-2): Check Appendix B to determine their Risk Group - then:	
	Risk Group 2 or 3 source. BL2
	Risk Group 4 source. . . . . .BL2, **IF** the pathogen genome is defective
	Risk Group 4 source. . . . . .BL4, **Otherwise**
• Animal Virus DNA source into Tissue Culture (Section III-D-3): Check Appendix B to determine their Risk Group- then:	
	Risk Group 2 virus source. BL2
	Risk Group 3 virus source. BL3
	Risk Group 4 virus source. BL4
• Transgenic *Animal*[2] Host (Section III-D-4):	
	Anything but > 2/3 eukaryotic virus genome. . . . . BL1-N (but IBC can boost this level based on the pathogenicity of the source organism)
	Viral vectors that don't transmit. BL1-N
	Everything else is a special case. . . . . .IBC decides

• Modified microorganisms into *Animals*: (Section III-D-4):	
	Any viable rDNA modified organism ≥BL2, BL2-N
• rDNA into *Animals*	
	Any rDNA that (except pieces >⅔ eukaryotic viral genome) BL1, BL1-N
	Everything else. . . . . IBC decides
• Whole *Plants*: (Section III-D-5)	
	Exotic[3] pathogen hosts that can damage the ecosystem. . . . . BL3-P or BL2-P+
	Plants with transmissible DNA from exotic pathogens that can damage the ecosystem. . . . . .BL3-P or BL2-P+
	Transmissible exotic pathogen hosts BL4-P
	Toxin DNA into plants. BL3-P
	Insect Pathogen DNA that can damage the ecosystem. . . . . BL3-P or BL2-P+
• Genes coding for vertebrate **Toxins** (Appendix F)	
	LD_{50} < 100 ng/kg . . . . . .Requires NIH & IBC approval
	100 ng/kg < LD_{50} < 100 µg/kg . . . . . Requires IBC approval & NIH notification. (except in E. coli-see below)
	100 ng/kg < LD_{50} < 1 µg/kg . . . . . .BL2 if in E. coli
	1 µg/kg < LD_{50} < 100 µg/kg. . . . . BL1 if in E. coli

[1]RAC can either take no action and transmit the protocol to the FDA or call for a full public review at one of its quarterly meetings.

[2]The purchase or transfer of transgenic rodents is exempt from the NIH guidelines.

[3]"Exotic" plant pathogens are defined as those not known to occur naturally in the US.

Some Exempt Classes are:

- DNA vaccines encoding epitopes from microbiological sources are generally exempt from the NIH Guidelines, even in human studies. This unusual exemption is found in Appendix VI-A.
- rDNA outside of living or viral organisms,
- r DNA that cannot replicate or express in vivo,
- DNA from a single nonchromosomal or viral source,
- The DNA source organism and the host organism are the same organism (but not released into the environment)
- The DNA source organism and the host organism normally exchange DNA (organisms that normally exchange are listed in Appendix A)

- DNA that does “not present a significant risk to health or the environment...”
- The rDNA is used exclusively in tissue culture and has <1/2 eukaryotic viral genome. There are exceptions to this rule (Appendix C-I-A). Check with the Biosafety Officer.
- Experiments using an E. coli host vector system in which the host does not contain conjugation proficient plasmids. There are some restrictions on the vectors used (Appendix C-II). BL1 containment is suggested.
- Experiments with Saccharomyces host-vector systems. There are some restrictions (Appendix C-III). BL1 containment is suggested.
- Experiments with Bacillus subtilis or B. icheniformis host-vector systems and in which reversion to spore formation is <10-7. There are some other restrictions (Appendix C-IV). BL1 containment is suggested.

“THREE SPECIALIZED GUIDELINE SECTIONS”

The NIH Guidelines recognize three non-laboratory classes of Biosafety containment and procedures; those in which genes are transferred into humans (Appendix M); those for plants (BL1-P through BL4-P, Appendix P) and those for animals (BL1-N through BL4-N, Appendix Q).

Humans

All Human Gene Transfer protocols are currently considered experimental. IBC has not yet established a Human Gene Therapy Advisory Committee to deal with human Gene Transfer studies.

IBC approval must await RAC (NIH Recombinant Advisory Committee) action. Depending on whether the study is deemed “novel” the RAC can either schedule a full examination of the protocol at one of its quarterly meetings or recommend sole FDA review.

Overcoming the regulatory hurdles involved in gaining approval for a human gene transfer study is not a task for the faint of heart. Beyond approvals from the Food and Drug Administration one has to get approval form the local Institutional Review Board (IRB) and the Biosafety Committee. In addition, RAC will evaluate novel protocols although it does not have approval power. These evaluations often involve the Principal Investigator’s appearance in Bethesda and aggressive questioning by members of the RAC.

For most people this process can be intimidating. Best to get help early through a commercial sponsor or a consultant.

Animals

Animal Biosafety levels are normally used to cover large animals such as cattle, swine, horses, poultry etc. IBC tends to use the same designations when considering safe practices with lower animals including rodents.

All animal experiments must be reviewed and approved by a local Institutional Animal Care and Use Committee (IACUC). These committees act under the US Department of Agriculture regulations. At Clemson University, the responsible committee is designated the Animal Research Committee (ARC).

Plants

Plant Biosafety levels are necessary when research plants are too big, too many or have growth requirements that cannot be covered by the standard Biosafety Levels. The plant guidelines cover microorganisms and small “animals,” particularly insects, such as arthropods. Plant-associated microorganism include pathogenic viroids, virusoids, viruses, bacteria, fungi, protozoans, as well as benign or beneficial microorganisms known to be associated with plants e.g. (Rhizobium, Pseudomonas).

When studies covered under the plant appendix are being discussed, IBC will include an expert in plant pests or containment.

It is of interest that the plant guidelines are not designed to directly protect humans from plant related recombinant DNA (i.e., the agents covered pose virtually no threat to humans or higher animals). Rather the guidelines are in place to protect the general ecosystem form serious disruption. Thus, procedures are designed to limit the spread of novel organisms form the experimental facility, not to protect the workers.

SUMMARY OF LABORATORY BIOSAFETY LEVELS

Biosafety Level	Risk Group	Practices and Techniques	Safety Equipment	Examples
BL1 **Basic Laboratory**	Individual risk: LOW Community risk: LOW	Standard Microbiological Practices.	None: primary containment provided by adherence to standard lab practices during open bench operations.	*E. Coli* K12, Continuous culture of cell lines, e.g., short term, long term culture of most non-primate mammalian tissue.
BL2 **Basic Laboratory** With Biosafety cabinets and other physical containment devices as required.	Individual risk: MODERATE Community risk: LOW	*Level 1 practices plus:* Lab coats, autoclaving of all biological waste preferred, limited access, biohazard warning signs on doors and equipment.	Partial containment (i.e., Class I or II Biosafety cabinets for procedures which produce aerosols.	Hepatitis B Virus, *Salmonella typhi*, Short term, long term culture of human tumor cell lines, culture of lymphoid lines carrying inducible EBV, many common human pathogens.
BL3 **Containment Laboratory** with special engineering and design features.	Individual risk: HIGH Community risk: MODERATE	*Level 2 practices plus:* Special protective clothing, controlled access through entrance room, biological waste must be autoclaved, preferably in facility.	Partial containment equipment used for all manipulations of infections materials, directional airflow.	Yellow fever, *M. tuberculosis*, Short term culture of tissue from non-human primates until cultures are known to be free of Herpes-virus *simiae* (B. virus)
BL4 **Maximum Containment Laboratory**	Individual risk: HIGH Community risk: HIGH	*Level 3 practices plus:* Entrance through change room. Complete change of clothing from street to laboratory gear, shower at exit. All wastes decontaminated on exit from facility.	Maximum containment equipment (i.e., Class III Biosafety cabinet or partial containment in combination with full-body, air-supplied positive-pressure personnel suit) used for all procedures and activities.	Ebola-Marburg Virus. Propagation of Herpes virus *simiae.* Smallpox.

SUMMARY OF ANIMAL FACILITY BIOSAFETY LEVELS

BSL	Agents	Practices	Safety Equipment (Primary Barriers)	Facilities (Secondary Barriers)
1	Not known to consistently cause disease in healthy human adults.	Standard animal care and management practices, including appropriate medical surveillance programs.	As required for normal care of each species.	Standard animal facility. No recirculation of exhaust air. Directional air flow recommended. Handwashing sink recommended.
2	Associated with human disease. Hazard: percutaneous exposure, ingestion, mucous membrane exposure.	ABSL-1 practices plus: Limited access. Biohazard warning signs. Sharps precautions. Biosafety manual. Decontamination of all infectious wastes and of animal cages prior to washing.	ABSL-1 equipment plus primary barriers: containment equipment appropriate for animal species; PPES: laboratory coats, gloves, face and respiratory protection as needed.	ABSL-1 facility plus: Autoclave available. Handwashing sink available in the animal room. Mechanical cage washer used.
3	Indigenous or exotic agents with potential for aerosol transmission; disease may have serious health effects.	ABSL-2 practices plus: Controlled access. Decontamination of clothing before laundering. Cages decontaminated before bedding removed. Disinfectant foot bath as needed.	ABSL-2 equipment plus: Containment equipment for housing animals and cage dumping activities. Class I or II BSCs available for manipulative procedures (inoculation, necropsy) that may create infectious aerosols. PPEs: appropriate respiratory protection	ABSL-2 facility plus: Physical separation from access corridors. Self-closing, double-door access. Sealed penetrations. Sealed windows. Autoclave available in facility.
4	Dangerous/exotic agents that pose high risk of life threatening disease; aerosol transmission, or related agents with unknown risk of transmission.	ABSL-3 practices plus: Entrance through change room where personal clothing is removed and laboratory clothing is put on; shower on exiting. All wastes are decontaminated before removal from the facility.	ABSL-3 equipment plus: Maximum containment equipment (i.e., Class III BSC or partial containment equipment in combination with full body, air-supplied positive-pressure personnel suit) used for all procedures and activities.	ABSL-3 facility plus: Separate building or isolated zone. Dedicated supply and exhaust, vacuum and decontamination systems. Other requirements outlined in BMBL-4.